Improved Synthesis of 2-substituted Adenosines

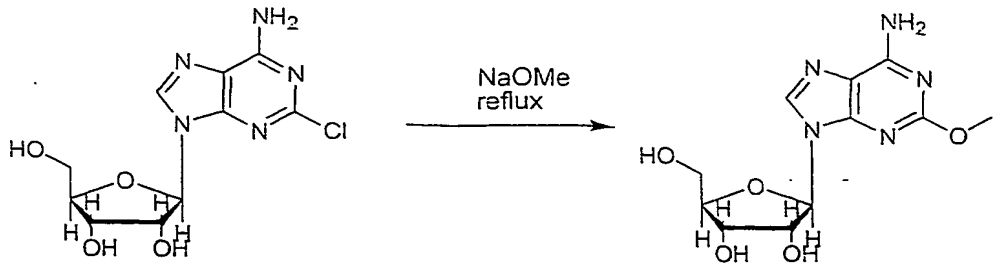
This invention relates to synthesis of 2-substituted adenosines, such as spongosine (2-methoxyadenosine), and synthesis of intermediates for use in the synthesis of such compounds.

The natural product spongosine was first isolated from a sponge, *Cryptotethia crypta,* collected off the Florida coast in 1945 (Bergmann and Feeney, J.Org. Chem. 1951, 16, 981; Ibid 1956, 21, 226). Spongosine was considered an unusual nucleoside in that it was not only the first methoxypurine to be found in nature but also one of the first O-methyl compounds to be isolated from animal tissues.

Several methods of synthesis of spongosine have been reported. One of the first of these to be published was by Bergmann and Stempien (J. Org. Chem. 1957, 22, 1575) in which spongosine was formed via coupling of chloromercuric 2-methoxyadenine to 2,3,5-tri-O-benzoyl-D-ribofuranosyl chloride. This simple coupling reaction provided a crude yield of spongosine of 31% which was then recrystallised from hot water to provide spongosine which exhibited a melting point of 191-191.5°C and an optical rotation of -43.5° (NaOH).

A variation on this theme was employed by Ojha *et al.* (Nucleosides and Nucleotides (1995, 14, 1889) who initially coupled 2-ethylthioadenine with a suitably protected ribose. Subsequent adjustments of the protecting groups and oxidation gave a substrate which was reacted with sodium methoxide to yield spongosine in a yield of 87% for the final step. The purity of the target spongosine after column chromatography, was proved by both elemental analysis and melting point (189-190°C).

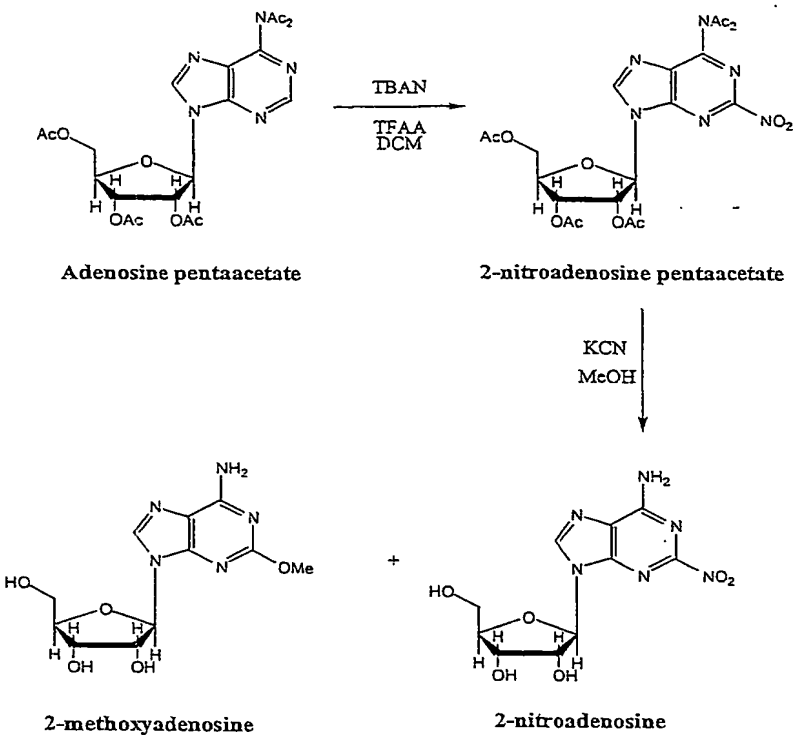
One of the most common methods of preparation of spongosine is via displacement of a 2-substituted chlorine atom by methoxide:



This methodology has been successfully applied by a number of groups to provide spongosine in varying yields and purity: Schaeffer *et al.*, J. Am. Chem. Soc. 1958, 80, 3738 (35% yield, mpt. 190-192°C); Bartlett *et al.*, J. Med. Chem. 1981, 24, 947 (yield and purity not quoted); Sato *et al.*, Synth. Proceed. Nucleic Acid Chem. 1968, 1, 264. However, this method suffers from the disadvantage that the 2-chloroadenosine starting material is difficult to synthesise, and consequently is expensive to produce.

Spongosine was reported by Cook et al. (J. Org. Chem. 1980, 45, 4020) as a by-product in the methylation reaction of isoguanosine by methyl iodide. Both the desired 1-methylisoguanosine and the spongosine were obtained in poor crude yields (19 and 30% respectively). The crude spongosine fragment was first purified by column chromatography on silica gel (eluent: chloroform/methanol) and then recrystallised from water to provide a sample which melted between 189-192°C (7% yield pure).

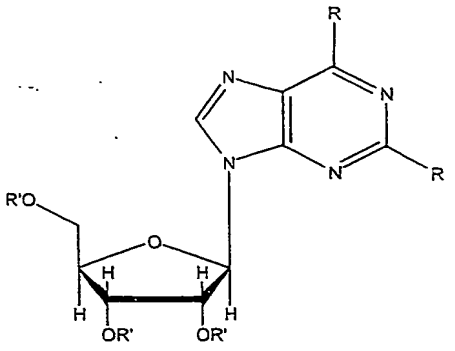
Deghati *et al* (Tetrahedron Letters 41 (2000) 1291-1295) and Wanner *et al* (Bioorganic & Medicinal Chemistry Letters 10 (2000) 2141-2144) describe formation of spongosine as a significant by-product in the synthesis of 2-nitroadenosine by treatment of 2-nitroadenosine pentaacetate with potassium cyanide in methanol. The 2-nitroadenosine was obtained in only 10% yield, and spongosine in 47% yield (Deghati *et al*). The 2-nitroadenosine pentaacetate was produced by nitration of adenosine pentaacetate with tetrabutylammonium nitrate/trifluoroacetic anhydride (TBAN/TFAA):



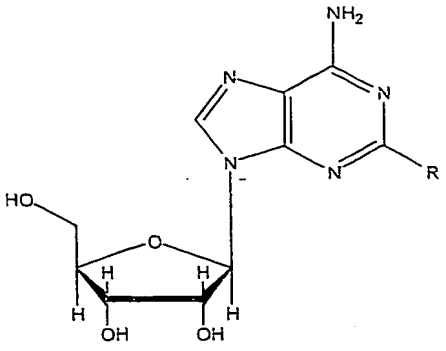
A disadvantage of this method is that the spongosine is not produced in high yield or purity. A further disadvantage is that it involves use of the toxic reagent potassium cyanide.

It is desired, therefore, to provide alternative methods of synthesis of spongosine and other 2-substituted adenosines, and of intermediates for use in the synthesis of these compounds. It is also desired to improve the yield and purity of the 2-substituted adenosines and intermediates obtained.

According to a first aspect of the invention there is provided a method of synthesis of a compound of formula I which comprises converting a compound of formula II to a compound of formula I:



II



I

wherein:

R is C_{1-6} alkoxy (straight or branched), a phenoxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy), a benzyloxy group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy), or a benzoyl group (unsubstituted, or mono-, or di-substituted by halo, amino, CF_3-, cyano, nitro, C_{1-6} alkyl, or C_{1-6} alkoxy);

R' = H, or a protecting group.

Preferably R is methoxy, ethoxy, propoxy, butoxy, pentyloxy, hexyloxy, phenoxy, benzyloxy, or benzoyl. More preferably R is methoxy.

It is preferred that the R groups of formula II are the same as each other, although in some circumstances it may be preferred that the R groups are different from one another.

It is preferred that the R' groups are the same as each other. However, in some circumstances it may be preferred that two or three different R' groups are used (for example one acetyl group and two benzoyl groups, or two acetyl groups and one benzoyl group).

Preferably the compound of formula I produced is isolated.

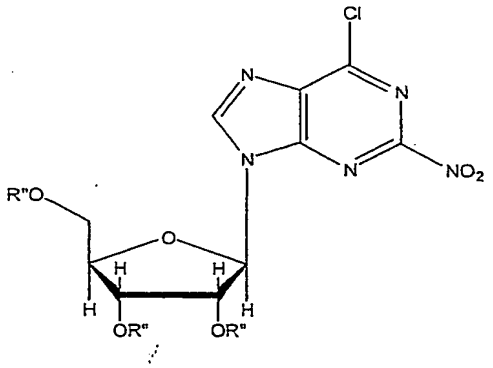
In some preferred embodiments of the invention R' is H, and the compound of formula II is aminated to form the compound of formula I. This may be achieved, for example by

heating the compound of formula II in a solution of ammonia (for example upto 80°C) and then cooling the solution to precipitate the compound of formula I. Preferably an aqueous solution of ammonia is used, although ammonia in methanol or ethanol may alternatively be used. Preferably the precipitate is then isolated, for example by filtration and washing.

Preferably the compound of formula II is 2,6-dimethoxy adenosine, and the compound of formula I is spongosine. A preferred method of converting 2, 6-dimethoxy adenosine to spongosine and isolating the spongosine produced is described in Step 5 of the Example below.

In other preferred embodiments of the invention R' is a protecting group. It is advantageous if the protecting group is removed under the same conditions that replace the R group at the 6-position of the purine component of the compound of formula II with an amino group. This allows the compound of formula II to be converted to the compound of formula I in a single reaction step. It is preferred that R' is a protecting group that can be removed by treatment with ammonia. Suitable protecting groups are acetyl and benzoyl.

Preferably methods of the first aspect of the invention further comprise converting a compound of formula III (preferably triacetoxy 2-nitro-6-chloroadenosine) to a compound of formula II:



III

wherein R'' is a protecting group, preferably acetyl or benzoyl.

It is preferred that the R'' protecting groups are the same as each other. However, in some circumstances it may be preferred that two or three different R'' protecting groups are used (for example one acetyl group and two benzoyl groups, or two acetyl groups and one benzoyl group).

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I which includes the step of converting a compound of formula III (preferably triacetoxy 2-nitro-6-chloroadenosine) to a compound of formula II.

There is also provided according to a further aspect of the invention a method of synthesis of a compound of formula II which comprises converting a compound of formula III (preferably triacetoxy 2-nitro- 6-chloroadenosine) to the compound of formula II.

Preferably the compound of formula II produced is isolated.

When the R' groups of the compound of formula II are protecting groups, it will be appreciated that they will usually be the same as each other, and the same as the R'' protecting groups of the compound of formula III. However, in some circumstances it may be desired that the R'' protecting groups are different to the R' protecting groups.

Preferably the compound of formula III (for example triacetoxy 2-nitro-6-chloroadenosine) is alkoxylated or benzoylated at the 2- and 6- positions to form the compound of formula II.

Preferably the compound of formula I is spongosine, and the compound of formula II is 2,6-dimethoxy adenosine.

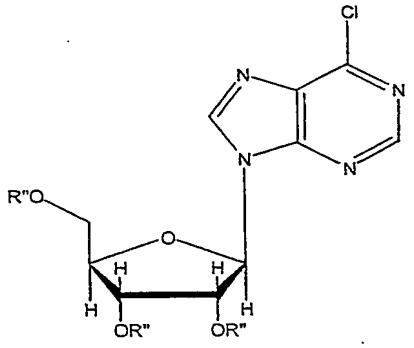
For embodiments of the invention in which the compound of formula II is 2,6-dimethoxyadenosine, and the compound of formula III is triacetoxy 2-nitro-6-chloroadensine , preferably the triacetoxy 2-nitro-6-chloroadensine is methoxylated at the 2- and 6- positions to form 2, 6-dimethoxy adenosine. This may be achieved, for example by contacting a solution of sodium methoxide in methanol with a solution of triacetoxy 2-nitro-6-chloroadenosine in dichloromethane (DCM) or chloroform.

An advantage of use of sodium methoxide/methanol as methoxylating reagent is that it is considerably less toxic than potassium cyanide/methanol used by Deghati *et al.*, and Wanner *et al.* Sodium methoxide/methanol also appears to give a higher yield of methoxylated product than potassium cyanide/methanol.

Preferably the 2, 6-dimethoxy adenosine is then isolated from the contacted solutions, for example by removing the methanol and DCM and purifying the 2, 6-dimethoxy adenosine by reverse phase column chromatography.

A preferred method of converting triacetoxy 2-nitro-6-chloroadenosine to 2, 6-dimethoxy adenosine and isolating the 2, 6-dimethoxyadenosine produced is described in Step 4 of the Example below.

Preferably methods of the first or further aspects of the invention further comprise converting a compound of formula IV (preferably triacetoxy 6-chloroadenosine) to a compound of formula III (preferably triacetoxy 2-nitro-6-chloroadenosine):



IV

wherein R'' is a protecting group, preferably acetyl or benzoyl. The R'' protecting groups should preferably be the same as the R'' protecting groups of formula III.

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I or a compound of formula II which includes the step of converting a compound of formula IV (preferably triacetoxy 6-chloroadenosine) to a compound of formula III (preferably triacetoxy 2-nitro-6-chloroadenosine).

Preferably the compound of formula III (for example triacetoxy 2-nitro-6-chloroadenosine) produced is isolated.

Preferably the compound of formula I is spongosine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably triacetoxy 6-chloroadenosine is nitrated at the 2-position to form triacetoxy 2-nitro, 6-chloroadenosine. Suitable nitrating reagents include tetrabutyl ammonium nitrate (TBAN), tetramethyl ammonium nitrate (TMAN) and sodium nitrate. For example a solution of triacetoxy 6-chloroadenosine may be contacted with a solution of TBAN and trifluoroacetic acid (TFAA), or TMAN and TFAA. Preferably a chlorinated solvent is used, such as DCM or chloroform.

Nitration of triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine using TBAN/TFAA in DCM is described in Deghati *et al.*, page 1292, lines 4-6 (although not in relation to synthesis of spongosine). TBAN/TFAA is also used by Deghati *et al.* to nitrate adenosine pentaacetate in the method of synthesis of spongosine disclosed in this document.

We have appreciated, however, that one of the principal reasons that spongosine is not produced in high yield and purity by the method of Deghati *et al.* is that TBAN and other tetrabutyl ammonium (TBA) salts contaminate the 2-nitroadenosine pentaacetate intermediate and interfere with subsequent synthesis steps.

According to the invention, the yield and purity of the spongosine product can be significantly improved if the amount of contaminating TBA salts is reduced. However, removal of these contaminants is problematic because they are amphiphilic and so cannot be completely removed by aqueous work-up.

We have found that the purity and yield of triacetoxy 2-nitro-6-chloroadenosine and subsequently produced 2, 6-dimethoxyadenosine and spongosine is surprisingly significantly improved by trituration of the triacetoxy 2-nitro-6-chloroadenosine from isopropanol, or preferably ethanol, and washing with a mixture of water and ethanol to

remove the TBA impurities.

We have appreciated that similar methods can be used to remove tetramethyl ammonium (TMA) impurities if tetramethyl ammonium nitrate (TMAN) is used as nitrating reagent instead of TBAN. Use of TMAN as nitrating agent may be preferred to use of TBAN because TMAN is easier to wash out with water than TBAN.

The TBA or TMA impurities are easier to remove from triacetoxy 2-nitro-6-chloroadenosine than from 2-nitroadenosine pentaacetate (used by Deghati *et al.*) because this latter compound decomposes in water. Thus, spongosine can be synthesised more easily in high yield and purity by using a triacetoxy 6-chloroadenosine intermediate.

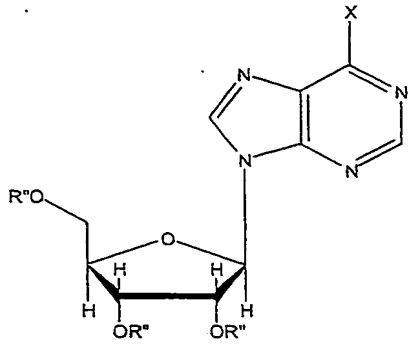
A preferred method of converting triacetoxy 6-chloroadenosine to triacetoxy 2-nitro-6-chloroadenosine and isolating the triacetoxy 2-nitro-6-chloroadenosine produced is described in Step 3 of the Example below.

We have appreciated that the above methods can be used to remove TBA or TMA impurities that contaminate compounds synthesised in other reactions by nitration of a substituted adenosine using TBAN or TMAN. The compounds may thereby be produced in higher purity, and the purity and yield of products produced by subsequent synthesis steps may be increased.

Thus, according to a further aspect of the invention there is provided a method of reducing the amount of TBA or TMA impurities contaminating a product formed by nitration of a substituted adenosine with TBAN or TMAN, which comprises triturating the product from isopropanol or ethanol, and washing the product with a mixture of water and ethanol.

There is also provided according to the invention a method of producing a nitrated substituted adenosine which comprises nitrating a substituted adenosine using TBAN or TMAN, and reducing the amount of TBA or TMA impurity contaminating the nitrated substituted adenosine.

Preferably the substituted adenosine is a compound of formula VI:



VI

wherein:

X is halo, preferably Cl, or -OMe; and

R'' is H, or a protecting group, preferably acetyl or benzoyl.

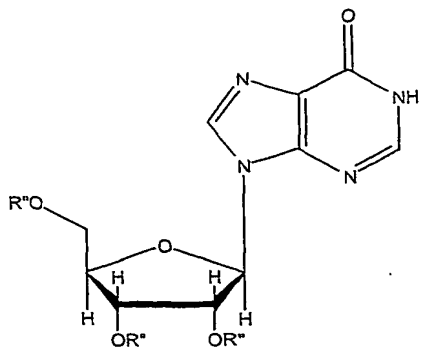
Preferably the amount of TBA or TMA impurity is reduced by triturating the nitrated substituted adenosine from isopropanol or ethanol, and washing the triturated product with a mixture of water and ethanol.

In general, a minimum of three washes with water/ethanol has been found to be required to remove a large proportion of the TBA or TMA impurities. However, five washes are generally carried out to ensure as much TBA or TMA impurity is removed as possible.

Instead of trituration, it may be possible to use column chromatography or reverse phase chromatography to reduce the amount of TBA or TMA impurity present.

The invention also provides nitrated, substituted adenosines produced by such methods.

Preferably methods of the first or further aspects of the invention further comprise converting a compound of formula V (preferably triacetoxy inosine) to a compound of formula IV (preferably triacetoxy 6-chloroadenosine):



V

wherein R'' is a protecting group, preferably acetyl or benzoyl. The R'' protecting groups should preferably be the same as the R'' protecting groups of formula IV (and/or formula III).

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I, a compound of formula II, or a compound of formula III, which includes the step of converting a compound of formula V (preferably triacetoxy inosine) to a compound of formula IV (preferably triacetoxy 6-chloroadenosine).

Preferably the compound of formula IV (for example triacetoxy 6-chloroadenosine) produced is isolated.

Preferably the compound of formula I is spongosine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably triacetoxy inosine is chlorinated to form triacetoxy 6-chloroadenosine. This may be achieved, for example by contacting DMF and thionyl chloride with a solution of triacetoxy inosine in choloroform. Instead of chloroform, DCM may be used as a solvent. Instead of thionyl chloride, $POCl_3$ may be used as chlorinating reagent.

Preferably the triacetoxy 6-chloroadenosine is isolated from the contacted DMF, thionyl chloride, and triacetoxy inosine solution, for example by removal of the DMF, thionyl chloride, and chloroform, partitioning of the resulting residue between DCM and aqueous

sodium bicarbonate, and washing of the separated organic phase with brine and drying over magnesium sulphate.

A preferred method of forming the triacetoxy 6-chloroadenosine from triacetoxy inosine, and isolating the triacetoxy 6-chloroadenosine produced is described in step 2 of the Example below.

Preferably methods of the first or further aspects of the invention further comprise converting inosine to a compound of formula V (preferably triacetoxy inosine).

According to a further aspect of the invention there is provided a method of synthesis of a compound of formula I, II, III, or IV, which includes the step of converting inosine to a compound of formula V (preferably triacetoxy inosine).

Preferably the compound of formula V (for example triacetoxy inosine) produced is isolated.

Preferably the compound of formula I is spongosine, and the compound of formula II is 2,6-dimethoxy adenosine.

Preferably inosine is acetylated or benzoylated to form the compound of formula V (preferably triacetoxy inosine). Acetylation of inosine to form triacetoxy inosine may be achieved, for example by contacting a suspension of inosine and catalytic DMAP in MeCN with Et_3N and acetic anhydride to form a solution before contacting the solution with methanol.

A preferred method of converting inosine to triacetoxy inosine and isolating the triacetoxy inosine produced is described in Step 1 of the Example below.

According to the invention there is also provided use of a compound of formula II, III (preferably triacetoxy 2-nitro, 6-chloroadenosine), IV (preferably triacetoxy 6-chloroadenosine), V (preferably triacetoxy inosine), or inosine in the synthesis of a compound of formula I.

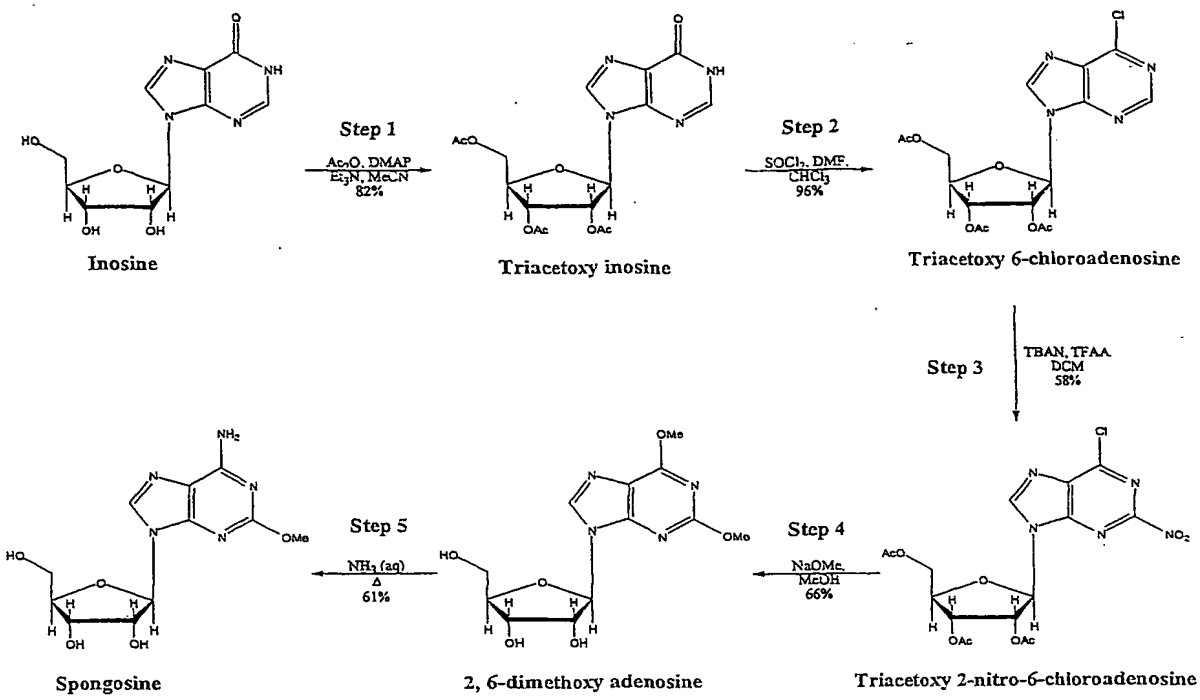
The invention further provides use of a compound of formula III (preferably triacetoxy 2-nitro, 6-chloroadenosine), IV (preferably triacetoxy 6-chloroadenosine), V (preferably triacetoxy inosine), or inosine in the synthesis of a compound of formula II.

Preferably the compound of formula I is spongosine and the compound of formula II is 2, 6-dimethoxy adenosine.

Methods of the invention allow synthesis of 2-substituted adenosines and intermediates for use in the synthesis of 2-substituted adenosines in high yield and purity, and do not require use of toxic reagents such as potassium cyanide.

Embodiments of the invention are now described by way of example only with reference to the accompanying Scheme 1 which shows the synthesis of spongosine from inosine.

Example



Scheme 1

Step 1

To a suspension of inosine (10g, 37.3mmol) and catalytic DMAP in MeCN (60mL) was added Et_3N (20mL, 143mmol) and acetic anhydride (12.5mL) and the resulting solution was stirred for 1h at ambient temperature before the addition of MeOH (5mL). After stirring for 5mins, the solution was concentrated *in vacuo* to yield a white solid which was washed with isopropyl alcohol to afford triacetoxy inosine (12.1g, 82%).

Step 2

To a solution of triacetoxy inosine (3.00g, 7.63mol) in $CHCl_3$ (25mL) was added DMF (1.80mL, 22.9mmol) and thionyl chloride (1.68mL, 22.9mmol) and the resulting solution was refluxed overnight before removal of the solvents *in vacuo*. The residue was then partitioned between DCM and aq. $NaHCO_3$ and the separated organic phase was washed with brine and dried over $MgSO_4$ to afford triacetoxy 6-chloroadenosine as a pale yellow oil (3.03g, 96%).

Step 3

To a solution of TBAN (4.43g, 14.5mmol) in DCM (15mL) at 0°C was added TFAA (2.05mL, 14.5mmol) and the resulting solution was stirred for 5mins, before the addition of triacetoxy 6-chloroadenosine (4g, 9.7mmol) in DCM (20mL). The resulting brown solution was stirred for 2.5h before being quenched with aq. $NaHCO_3$, extracted into DCM and dried over $MgSO_4$. Purification *via* trituration from EtOH yielded triacetoxy 2-nitro, 6-chloroadenosine as a pale yellow solid which was washed with 1:1 EtOH/water to afford 2.57g, 58%.

Step 4

To a solution of NaOMe (590mg, 10.9mmol) in MeOH (10mL) was added dropwise a solution of triacetoxy 2-nitro, 6-chloroadenosine (1g, 2.19mmol) in DCM (10mL) and the resulting red solution was stirred overnight. The solvents were then removed *in vacuo* and the product was purified by reverse phase column chromatography (gradient 30-70% MeOH/water) to afford 2,6-dimethoxy adenosine as a white solid (447mg, 66%).

Step 5

A solution of 2,6-dimethoxy adenosine (697mg, 3.23mmol) in aq. NH_3 was heated in a sealed tube at 80°C for 26h. The solution was then cooled and the resulting white precipitate was filtered and washed with cold water to afford 2-methoxy adenosine (406mg, 61%).

In other preferred embodiments benzoyl protecting groups may be used instead of the acetyl protecting groups shown.